

WHAT IS CLAIMED IS:

1. A device comprising:

a microfluidic substrate comprising at least one pathway for sample flow; and

at least one thermal transfer member which is capable of cycling between at least two temperatures, said at least one thermal transfer member being adapted to bring at least a portion of said sample pathway to said at least two temperatures while a sample is continuously flowing along said at least a portion of said sample pathway.

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2. The device of Claim 1, further comprising a force supplying member operably linked to said at least one pathway for sample flow wherein said force supplying member applies a force to said sample such that said sample travels along said at least one pathway.

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3. The device of Claim 2, further comprising a sample supplier which supplies a sample to said at least one pathway.

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4. The device of Claim 3, further comprising at least one inlet basin positioned at a first end of said at least one pathway such that said sample supplier supplies said sample to said inlet basin and said sample travels from said inlet basin to said at least one pathway.

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5. The device of Claim 4, further comprising at least one outlet basin positioned at a second end of said pathway.

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6. The device of Claim 5, further comprising at least one reagent supplier positioned between said inlet basin and said outlet basin.

7. The device of Claim 6, wherein said device comprises a plurality of said pathways.

8. The device of Claim 7, wherein said pathways comprise channels arranged in parallel.

9. The device of Claim 8, wherein the force generated by said force supplying member is pressure.

5            10. The device of Claim 1, wherein said microfluidic substrate consists essentially of silicon.

*Sub 2*            11. The device of Claim 1, further comprising a detector for measuring a physicochemical property of said biological sample.

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*Sub A2*            ~~12. The device of Claim 1, wherein said thermal transfer member comprises a metal bar in fluid communication with a plurality of water sources containing water at said at least two temperatures, said metal bar being in thermal communication with said at least a portion of said sample pathway.~~

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13. A method for conducting a biochemical or chemical process comprising:  
                 cycling at least a portion of at least one sample flow pathway  
between at least two temperatures while a sample comprising the reagents for  
said biochemical or chemical process is flowing through said at least a  
20            portion of said at least one sample flow pathway.

14. The method of Claim 13, wherein said sample flow pathway is located  
on a microfluidic substrate.

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15. The method of Claim 14, wherein said sample flow pathway is in thermal  
communication with at least one thermal transfer member which cycles between said  
at least two temperatures while said sample is continuously flowing through said at  
least a portion of said at least one sample flow pathway.

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16. The method of Claim 15, wherein said thermal transfer member cycles  
through said at least two temperatures a plurality of times while said sample is  
continuously flowing through said at least a portion of said at least one sample flow  
pathway.

17. The method of Claim 16, wherein said thermal transfer member cycles through said at least two temperatures from about 2 to about 35 times while said sample is continuously flowing through said at least a portion of said at least one sample flow pathway.

18. The method of Claim 16, wherein at a portion of a plurality of sample flow pathways are simultaneously cycled between said at least two temperatures while a plurality of samples are simultaneously flowing through said sample flow pathways.

19. The method of Claim 18, wherein said biochemical or chemical reaction comprises a nucleic acid amplification procedure.

20. The method of Claim 19, wherein said nucleic acid amplification procedure comprises polymerase chain reaction.

21. The method of Claim 19 further comprising determining the identity of at least one polymorphic nucleotide in the product of said nucleic acid amplification procedure.

22. A process for carrying out biochemical protocols on at least one sample, comprising:

feeding at least one channel with a continuous flow of a solution containing at least one sample;

injecting at least one reagent from a reagent reservoir into said channel, thereby mixing said sample and said reagent; and

transferring heat between at least one thermal support and at least one temperature regulated portion of said at least one channel.

23. The process according to Claim 22, wherein said feeding comprises applying a pressure difference between a feed basin of said at least one channel and an outlet basin of said at least one channel.

24. [REDACTED] process according to Claim 22, further comprising detecting at least one physicochemical parameter of said sample in said at least one channel.

25. The process according to Claim 22, wherein a temperature of said  
5 solution is adjusted to a predetermined level when said solution runs through said at  
least one temperature regulated portion of said at least one channel.

26. The process according to Claim 22, further comprising cycling said at least one thermal support through at least two different temperatures.

27. The process according to Claim 26, wherein said cycling is repeated 1 to 35 times while solution is running through said at least one portion of said at least one channel.

15                    28. The process according to Claim 22, wherein a plurality of samples  
separated by separators are sequentially introduced into said at least one channel.

29. The process according to Claim 22, wherein said feeding, said injecting, and said transferring are carried out simultaneously on a plurality of channels arranged in parallel.

30. A process for carrying out in continuous flow at least one temperature cycle on a solution containing at least one sample, comprising:

25       feeding at least one channel with a continuous flow of said solution;  
      running said solution through at least one temperature regulated zone;  
      and  
      cycling said at least one temperature regulated zone successively through a temperature cycle of at least two temperatures in a predetermined temporal series, such that the solution undergoes said temperature cycle at

30       least once in running through the at least one temperature regulated zone once.

31. The process according to Claim 30, further comprising detecting at least one physicochemical parameter of said sample in said channel.

32. The process according to Claim 30, wherein said feeding comprises applying a pressure difference between a feed basin of said at least one channel and an outlet basin of said at least one channel.

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33. The process according to Claim 30, wherein said feeding is sequentially repeated with a plurality of samples separated by separators.

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34. The process according to Claim 30, wherein said feeding, said running and said cycling are carried out simultaneously on a plurality of channels arranged in parallel.

35. A process for amplifying nucleic acids, comprising:

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a) mixing at least one sample comprising said nucleic acids with reagents which are suitable for amplifying nucleic acids to form at least one reaction mixture;

b) feeding at least one channel with a continuous flow of said at least one reaction mixture;

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c) running said at least one reaction mixture through at least one temperature regulated zone; and

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d) cycling said temperature regulated zone through a temperature cycle of at least two temperatures in a predetermined temporal series, wherein the at least two temperatures, a duration of the temperature cycle, and a rate of said running are preselected such that said at least one nucleic acid sample undergoes a denaturation-hybridization-elongation cycle one or more times while flowing through said at least one temperature regulated zone.

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36. The process according to Claim 35, wherein said feeding comprises applying a pressure difference between a feed basin of said at least one channel and an outlet basin of said at least one channel.

37. The process according to Claim 35, wherein said channel is formed in a microfluidic substrate.

38. The process according to Claim 35, wherein said microfluidic substrate consists essentially of silicon.

5           39. The process according to Claim 35, in which said feeding is sequentially repeated with a plurality of nucleic acid samples separated by separators.

40. The process according to Claim 35, in which steps a), b), c) and d) are carried out simultaneously on a plurality of channels arranged in parallel.

10           41. A process for identifying in continuous flow at least one nucleotide in at least one target nucleic acid, comprising:

          a)     feeding a channel with a continuous flow of a solution comprising said at least one target nucleic acid;

15           b)     injecting a microsequencing reagent comprising a microsequencing buffer, at least one microsequencing primer, at least one ddNTP and a polymerase into said channel, thereby mixing said nucleic acid solution and said reagent;

          c)     running the solution through at least one temperature regulated zone in such a way as to produce at least one cycle comprising denaturation of said at least one target nucleic acid, hybridization of said nucleic acid with said at least one microsequencing primer, and incorporation of a ddNTP which is complementary to the nucleotide to be identified at a 3' end of said primer; and

20           d)     identifying the nucleotide which has been incorporated at the 3' end of the microsequencing primer.

42. The process according to Claim 41, wherein said feeding comprises applying a pressure difference between a feed basin of said channel and an outlet basin of said channel.

43. The process according to Claim 41, further comprising amplifying said at least one target nucleic acid prior to performing said method for identifying at least

one nucleotide wherein said at least one target nucleotide sequence is amplified using a method comprising:

a) mixing at least one sample comprising said nucleic acids with reagents which are suitable for amplifying nucleic acids to form at least one reaction mixture;

b) feeding at least one channel with a continuous flow of said at least one reaction mixture;

c) running said at least one reaction mixture through at least one temperature regulated zone; and

d) cycling said temperature regulated zone through a temperature cycle of at least two temperatures in a predetermined temporal series, wherein the at least two temperatures, a duration of the temperature cycle, and a rate of said running are preselected such that said at least one nucleic acid sample undergoes a denaturation-hybridization-elongation cycle one or more times while flowing through said at least one temperature regulated zone.

44. The process according to Claim 41, wherein the ddNTPs are labelled with fluorophores and wherein the fluorescence of the incorporated ddNTP is detected.

45. The process according to Claim 44, in which said feeding, said injecting and said running are carried out simultaneously on a plurality of channels arranged in parallel.

46. A process for detecting in continuous flow at least one nucleotide in at least one target nucleic acid, comprising:

a) feeding a channel with a continuous flow of a solution containing at least one target nucleic acid;

b) injecting the reagent for amplifying a region of the at least one target nucleic acid which carries at least one nucleotide to be detected into said channel from a first reagent reservoir;

running the solution through at least one temperature regulated zone in such a way that the nucleic acid undergoes a denaturation-hybridization-elongation cycle one or more times;

d) injecting the reagent for purifying the amplification product into said channel from a second reagent reservoir;

e) running the solution through at least one temperature regulated zone to carry out a purification reaction;

f) injecting the microsequencing reagent comprising the microsequencing buffer, at least one microsequencing primer, at least one ddNTP and a polymerase into said channel from a third reagent reservoir;

g) running the reaction mixture through at least one temperature regulated zone in such a way as to produce at least one cycle comprising the denaturation of the target nucleic acid, the hybridization of said nucleic acid with the at least one microsequencing primer, and the incorporation of the ddNTP which is complementary to the nucleotide to be detected, at the 3' end of said primer; and

h) detecting at least one ddNTP which is incorporated at the 3' end of the microsequencing primer.

47. The process according to Claim 46, wherein said feeding comprises applying a pressure difference between a feed basin of said channel and an outlet basin of said channel.

48. The process according to Claim 46, wherein in steps c) and e), the temperature regulated zone is brought successively to at least two temperatures in a temporal series which forms at least one cycle.

49. The process according to Claim 46, wherein the ddNTPs are labelled with fluorophores, and wherein in step h) the fluorescence of the incorporated ddNTP is detected.

50. The process according to Claim 46, wherein the reagent for the purification comprises an exonuclease and an alkaline phosphatase.



51. [REDACTED] process according to Claim 46, where [REDACTED] steps a), b), c), d), e), f), g) and h) are carried out simultaneously on a plurality of channels arranged in parallel.

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